

AMENDMENTS TO THE SPECIFICATION

The following is a marked-up version of the Specification's replacement paragraphs pursuant to 37 C.F.R. §1.121(b), showing changes made herein to the previous version of record of the Specification. Underlining denotes added text while strikeout denotes deleted text.

Please replace the paragraph beginning at page 5, line 22, with the following rewritten paragraph:

C Figures 1A-1B shows the nucleic acid (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequence of the mutD mutD gene. Illustrative examples of mutations of the mutD mutD gene are provided.

Please replace the paragraph beginning at page 5, line 24, with the following rewritten paragraph:

C2 Figures 2A-2B provides the nucleic acid sequences (SEQ ID NO:3 and 5, respectively) for the enzyme 1,3-propanediol dehydrogenase (PDD).

Please replace the paragraph beginning at page 5, line 26, with the following rewritten paragraph:

C3 Figures 3A-3B provides the amino acid sequences (SEQ ID NO:4 and 6, respectively) for the enzyme 1,3-propanediol dehydrogenase (PDD).

Please replace the paragraph beginning at page 10, line 22, with the following rewritten paragraph:

C4 In illustrative embodiments disclosed herein, a mutated mutD mutD gene residing on a plasmid was introduced via recombinant techniques into E.coli or E.blatte E. coli or E. blattae. The E.coli or E.blatte E. coli or E. blattae cell was then cultured under conditions suitable for growth for a time sufficient for at least 20 doublings and up to at least about 2000 doublings under conditions of selective pressure. In one example, E.coli E. coli was grown under conditions of increased temperature or in the presence of DMF and in another E.blattae E. blattae was growth in the presence of solvent, such as DMF or 1,3 propanediol. As a result, E.coli E. coli was evolved into a microorganism capable of growing at temperatures up to about 48° C or in the presence of 80g/l DMF. E.coli E. coli evolved to grow at elevated temperatures

also became auxotrophic for three amino acids, Cys/Met, Asp/Asn and Pro. E. blattae E. blattae was evolved into a microorganism capable of growing anaerobically in the presence of at least 105 g/l 1,3-propanediol and which comprised genetic changes in at least one catalytic activity associated with 1,3 propanediol production, 1,3-propanediol dehydrogenase, shown in Figure 3 (SEQ ID NO:4).

Please replace the paragraph beginning at page 12, line 9, with the following rewritten paragraph:

Mutator genes of the present invention include but are not limited to, mutations of the DNA repair genes mutD, mutT, mutY, mutM, mutH, mutL, mutS or mutU mutD, mutT, mutY, mutM, mutH, mutL, mutS or mutU or their homologues in other microorganisms. A description of the DNA repair genes are disclosed in Miller, *supra*; mutD mutD is disclosed in Maki et al., 1983, Proc. Natl. Acad. Sci., U.S.A. 80, 7137-7141 (GenBank accession number K00985.1 GI: 147678 and Figure 1); B. subtilis mutS and mutL B. subtilis mutS and mutL are disclosed in Ginetti et al., 1996, Microbiology, Aug, 142 (Pt 8): 2021-9; Streptococcus pneumoniae Streptococcus pneumoniae hex B repair gene, mutL of Salmonella typhimurium and PMS1 of Saccharomyces cerevisiae mutL of Salmonella typhimurium and PMS1 of Saccharomyces cerevisiae are disclosed in Prudhomme et al., 1989, J. Bacteriology, Oct; 171 (10): 5332-8; Streptococcus pneumoniae hexA and mutS of Salmonella typhimurium and E. coli Streptococcus pneumoniae hexA and mutS of Salmonella typhimurium and E. coli are disclosed in Priebe et al., J. Bacteriol., 1988, Jan; 170(1): 190-6 and Prudhomme et al., 1991, J. Bacteriol. Nov; 173(22): 7196-203; human mutS mutS homologue, hMSH2 hMSH2, and human MutL MutL homologue, hMLH1 hMLH1, are disclosed in Macdonald et al., 1998, Heptology, Jul 28(1):90-7; the mut-1 of Neurospora mut-1 of Neurospora is disclosed in Dillon et al., 1994, Genetics, Sep 138(1):61-74 and yeast homologues of mutL and mutS mutL and mutS are disclosed in WO 97/15657. The methods of the present invention comprises the use of at least one of the mutant DNA repair genes and may involve the use of more than one. It is preferred that a mutator gene be dominant to the wild type gene of the microorganism such that mutations are introduced into the genome of the microorganism. In a preferred embodiment, the mutator gene is a mutation of the mutD mutD gene. The nucleic acid and amino acid sequence for mutD is shown in Figure 1 (SEQ ID NO:1 and 2, respectively). One particular mutD mutD mutation, mutD5 mutD5, is disclosed in Takano, K., et al., (1986, Mol Gen Genet 205, 9-13, Structure and function of dnaQ and mutD dnaQ and mutD mutators of Escherichia

coli). Strain CSH116 was obtained as disclosed in Miller, J. H. (1992, A Short Course in Bacterial Genetics). This strain is reported to carry the mutD5 mutD5 allele. The mutD mutD gene in this strain was found to be very different from the published mutD5 mutD5. The mutD mutD gene from strain CSH116 is designated herein as mutD5' mutD5'. Table I gives the mutations found in mutD5 and mutD5'. mutD5 and mutD5'. Further mutations in mutD mutD which result in increased levels of mutation frequency were identified recently in Taft-Benz, S. A. et al., (1998, *Nucl. Acids Res.* 26, 4005-4011, Mutational analysis of the 3'-5' proofreading exonuclease of Escherichia coli DNA polymerase III). Table I describes various mutD mutD mutations useful in the present invention. Table II describes various promoters used with the mutD mutD mutations and Table III describes mutator plasmids and the range of available mutation frequencies in *E.coli* *E. coli*.

Please replace the Table on page 14, with the following rewritten Table:

Name	Mutations
wild type	ATGACCGCTATG...(SEQ ID NO:7)
pOS100	TTGA-CGCTTG...(SEQ ID NO:8)
pOS101	GTGACCGCTGTG...(SEQ ID NO:9)
pOS102	GTG-CCGCTGTG...(SEQ ID NO:10)
pOS104	TTGACCGCTTG...(SEQ ID NO:11)
pOS105	GTGACCGCTGTGAGCACTT(G)CAATTACACGCCAGATCGTTCTCGATACCGAA AT(C)...(SEQ ID NO:12)
pOS106	GTGACCGCT-TG...(SEQ ID NO:13)

Please replace the paragraph beginning at page 17, line 17, with the following rewritten paragraph:

Nucleic acid encoding a mutator gene can be isolated from a naturally occurring source or chemically synthesized as can nucleic acid encoding a protein or enzyme. Sources for obtaining nucleic acid encoding DNA repair genes mutD, mutT, mutY, mutM, mutH, mutL, mutS or mutU mutD, mutT, mutY, mutM, mutH, mutL, mutS or mutU is provided in Section II. Figure 1 provides the nucleic acid (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) for mutD and Table I and III provide preferred mutations for mutD mutD and the mutation rates obtained for each construct. Once nucleic acid encoding a mutator gene is obtained, plasmids or other expression vectors comprising the mutator gene may be constructed using techniques well known in the art. Molecular biology techniques are disclosed in Sambrook et al., Molecular

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Biology Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) and Brown, T. Current Protocols in Molecular Biology, Supplements 21, 24, 26 and 29. Nucleic acid encoding a mutator gene is obtained and transformed into a host cell using appropriate vectors. A variety of vectors and transformation and expression cassettes suitable for the cloning, transformation and expression in bacteria are known by those of skill in the art.

Please replace the paragraph beginning at page 19, line 29, with the following rewritten paragraph:

C S
mutD and mutD5' genes were amplified by PCR using mutd1 (5'-CGCCTCCAGCGCGACAATAGCGGCCATC-3' SEQ ID NO:14) and mutd2 (5'-CCGACTGAACCTACCGCTCCGCCTTG-3' SEQ ID NO:15) primers from genomic DNA of E. coli and E. coli CSH116 (Miller 1992), respectively. The PCR products were cloned into pCR-Blunt vector (Invitrogen, Carlsbad, CA). Plasmids from clones with the correct orientation were isolated and digested with SmaI-HindIII restriction enzymes. The overhang ends were filled using T4 polymerase and cloned into pMAK705 plasmid digested with SmaI-PvuII. The ligation products were transformed into JM101 competent cells. The resulted plasmids had the temperature-sensitive origin of replication, carried kanamycin resistance marker and were named pMutD-71 (control plasmid, wild type genotype) and pMutD5-61 (mutator plasmid).

Please replace the paragraph beginning at page 28, line 32, with the following rewritten paragraph:

C a
The dhaT genes were amplified by PCR from genomic DNA from E. blattae as template DNA using synthetic primers (primer 1 and primer 2) based on the K. pneumoniae dhaT sequence and incorporating an XbaI XbaI site at the 5' end and a BamHI BamHI site at the 3' end. The product was subcloned into pCR-Blunt II-TOPO (Invitrogen). The cloning dhaT were then sequenced was standard techniques.

The results of the DNA sequencing are given in SEQ ID NO:34 and SEQ ID NO:42.

Primer 1

5' TCTGATACTGGATCCTCAGAATGCCTGGCGGAAAAT3' (SEQ ID NO:1446)

Primer 2

5' GCGCCGTCTAGAATTATGAGCTATCGTATGTTGATTATCTG3' (SEQ ID NO:1547)

As will be readily understood by the skilled artisan, nucleic acid sequence generated via PCR methods may comprise inadvertent errors. The present invention also encompasses nucleic acid encoding PDD obtainable from E. blattae E. blattae having ATCC accession number PTA-92.

After page 29, please insert the Sequence Listing on pages 1-7, enclosed herewith.